T wave Spectral Analysis As A Marker For An Increased Risk Of Sudden Cardiac Death Using an in vivo Canine Model

A Thesis

Presented in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science in Engineering *with distinction* in the College of Electrical and Computer Engineering and the University Honors Program

By

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Spring 2008

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To my family, for their love and support through my education career.

ACKNOWLEDGEMENTS

Most importantly, I would like to thank Dr. Bradley Clymer for his help, patience, and guidance throughout this project. Without his support as a professor and mentor, this undertaking would not have been possible. In addition, I would like to thank Dr. George Billman for letting me shadow his medical experiments and have access to his data. Without these data this project would not have been possible. My interest of combining engineering and medicine was fueled by the opportunity to assist Dr. Billman with surgery and his experimental model.

Special thanks also go to Carlos L. del Rio and Dr. Clymer's Research Group. Work with Carlos involved collaboration to develop a better understanding of EKG analysis tools and data acquisition software. Carlos acted as my MatLab guru during collaboration to develop and debug the code used for T wave detection and other various distinction project landmarks. The Matlab file used to retrieve data from the .ACQ files was created and supplied by Carlos. Without Carlos's ability to bridge the gap between the engineering world and the medical world, this project would have been lost in translation.

Dr. Clymer's Research group was actively involved in monitoring the progress of the project. During my quarterly presentations the group participated in brainstorming sessions. Members continuously challenged my knowledge asking detailed questions and providing constructive feedback. Group suggestions and criticism, helped guide the project to the results outlined in this document.

Finally, Thank you to both the University Honors and Scholars Center and The College of Engineering. Without the programming, funding, and mentorship I received from these two groups of individuals, I would not have been able to pursue my academic interests – represented by this thesis.

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CHAPTER 1

Introduction

300,000 to 500,000 annual deaths in the United States are attributed to sudden cardiac failure [1]. While remaining the leading cause of death in industrially developing countries, effective diagnosis remains an elusive target. Although there are current diagnostic tools that can identify patients at risk for sudden death, these techniques are not without limitations. For example, heart rate variability (a marker for cardiac vagal tone), only analyzes the beat to beat variability in R-R interval of the electrocardiogram (EKG). This type of analysis neglects valuable information that may be contained in other portions of the EKG, particularly the T wave. The T wave may provide information about alterations in repolarization of the cardiac tissue that have been linked to arrhythmia formation. Therefore, a comprehensive analysis of the T wave (both changes in peak and shape) could provide novel approach for the identifications of patients at risk for cardiac related deaths.

A well established canine model of ventricular fibrillation (VF) provided a data set collected over the last 25 years that can be used to analyze and to identify factors that could be related to the risk for VF. This comprehensive and complete data set, along with each dog's continuous electrocardiogram (EKG), provides many examples of animals that were either susceptible or resistant to VF. Using these data, a spectral analysis of the

dog's EKG T wave variation can provide a new noninvasive, quick and effective method for determining a canine's susceptibility to VF.

This knowledge can then be applied in other aspects of diagnosis and treatment ranging from future anti-arrhythmic drug development to a less costly, more effective, internal defibrillator along with simpler methods of identifying patients at risk of cardiac disease and tailoring an appropriate therapy for each patient [1].

1.1 Heart electrochemical gradients

Ventricular Fibrillation (VF) is an abnormality in cardiac electrical activity that results in rapid, unsynchronized contractions of the ventricles such that heart is unable to pump blood to the rest of the body. This lack of blood flow leads to irreversible damage of the brain, heart and other tissue leading ultimately to death. To better understand VF, we look at the electrical properties of the heart.

The heart can be considered a simple circuit controlled by action potentials that result from characteristic changes in the electrochemical gradients of different ions. The ventricular myocardial membrane potential of a resting heart (not contracting, not de-polarizing or re-polarizing) during the T-P interval is -90mV. The ions responsible for spreading excitation through the heart muscles are potassium (K^+), sodium (Na^+), and calcium (Ca^{2+}). At rest the permeability to potassium ions is high while the sodium and calcium permeabilities are low. Changes in these permeabilities produce the cardiac action potential. Depolarization of the ventricle action potentials results from sodium ions flowing (increase in sodium permeability) into the heart resulting in a membrane potential of approximately 10mV. Once a critical voltage (threshold) has been reached the sodium permeability decreases as the result of inactivation or closure of Na^+ channels. (Note "closing" means the flow is much more limited, but is not entirely stopped)

The closing of these channels creates a plateau at approximately 0mV. Next, the permeability of calcium into the myocardial cell increases as the result of opening calcium

channels. The plateau is maintained by the restriction of potassium leaving the cell (closure of potassium channels) to match the calcium entering the cell. The result of calcium flow into the cell, balances the positive potassium flow out of the cell to maintain a plateau membrane permeability of 0mV.

This plateau is responsible for the refractory period of the heart, the length of time at which the heart cannot generate new action potentials. It is by this process the healthy heart avoids contracting rapidly at close intervals. While the pacemaker effect of the heart is determined by the spontaneous diastolic depolarization of specialized "pacemaker" cells, for simplicity think of this plateau as a cardiac regulator. The plateau prevents the heart from immediately re-contracting, allowing the atria and the ventricles to operate separately and rhythmically. Most importantly, if this plateau did not exist the heart would not be able operate properly. Finally, repolarization (i.e. the return to resting potential) results from the closure of the calcium channels and the opening of potassium channels, such that fewer positive ions enter (calcium ions) the cell than leave (potassium ions) thereby returning the membrane potential to -90mV. The membrane potential recording from a ventricular muscle cell and the permeabilities of potassium, sodium, and calcium during action potentials can bee seen in Figure 1 and Figure 2 respectively [2]

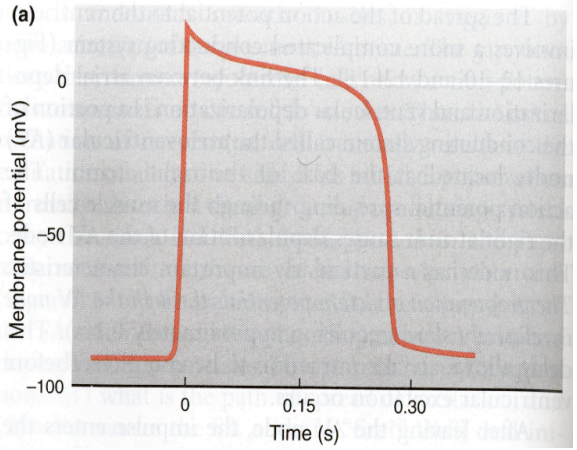

Figure 1: Membrane potential recording from a ventricular muscle cell

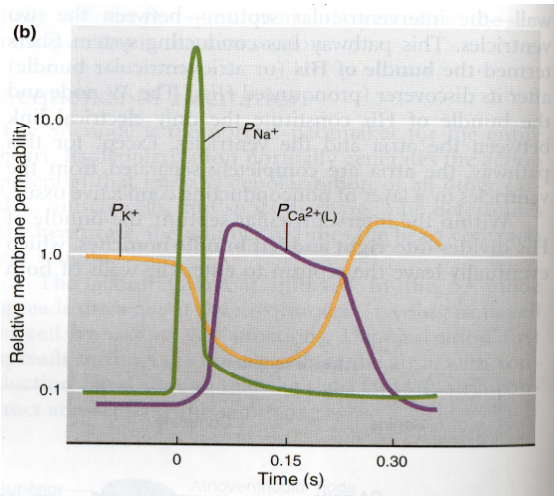

Figure 2: Simultaneously measured permeabilities (P) to potassium, sodium, and calcium during the action potential of figure 1

The action potentials created by these electrochemical gradients help generate a baseline pacemaker effect for the heart. The pacemaker potential is the spontaneous depolarization during diastole of specialized cells usually located in the right atrium at or near the sino-atrial node. This pacemaker depolarization, caused by electrochemical gradients, can be modified by sympathetic neural activity (increased discharge rate) or parasympathetic activity (decreased discharge rate). Ventricular action potentials can be influenced by factors that alter the ion permeability and/or the electrochemical gradient. These factors include myocardial ischemia and alterations in the autonomic neural regulation of the heart. Abnormalities in these ions can lead to cardiac rhythm disorders including ventricular fibrillation

1.2 Heart analyzers

Analyzing an EKG enables a cardiologist to identify cardiac arrhythmias ranging from cardiac arrest, ventricular tachycardia, ventricular fibrillation, to simple pre-ventricular contractions (PVCs). The EKG can also provide information as to factors that have been linked to a greater risk for rhythm disorders. Heart rate variability (HRV) as an index of cardiac vagal tone is often used to predict which patients have the greatest risk for cardiac arrhythmias.

Heart rate variability provides an indirect measure of cardiac parasympathetic response to the activity of the vagus nerve (i.e. cardiac vagal tone). The best way to understand this concept is through example. This heart rate variability is associated with changes in respiration and is often called a respiratory sinus arrhythmia. During inspiration, cardiac vagal activity is inhibited resulting in increase in heart rate while during expiration cardiac vagal activity increases, decreasing heart rate. This beat to beat variation in heart rate demonstrates that the heart can respond rapidly to changing environmental demands and is taken as sign of good cardiac health.

HRV has been significantly correlated with the autonomic nervous system and cardiovascular mortality. HRV describes variation of instantaneous heart rate for R-R intervals in an EKG. Figure 3 shows a varying R-R interval. [3]

Figure 3: EKG demonstrating Heart Rate Variability

HRV is related to changes in the sinus (atrial pacemaker) rhythm but ventricular arrhythmias are excluded from HRV analysis proving unhelpful when analyzing ventricular fibrillation. As such, HRV does not provide a complete measure of how the heart response to arrhythmias. HRV is, however, used to provide a marker of the general electrical stability of the heart. A high HRV indicates that the heart can respond to changing demands thereby having a better electrical stability than patients with low HRV. This high heart rate variability correlates to a high vagal tone and a high vagal tone increases the electrical stability of the heart making it less likely that stress will provoke VF.

T wave analysis can compensate for shortcomings of HRV analysis by providing information about repolarization of the heart. Heterogeneity of repolarization (regional differences in repolarization) increases the risk for the formation of arrhythmias, which are pre-cursors to VF. T wave analysis could provide information about these repolarization abnormalities. The current method used to determine a heart's probability of going into VF is to analyze the alternating peaks of the T waves. T wave peak

alternans are analyzed for variations and sinusoidal rhythms. It is hypothesized if variations exist then a canine will be susceptible to ventricular fibrillation. [4]

Studies have shown that canines (and hypothesized in humans) are genetically predisposed as resistant or susceptible to VF. A susceptible canine will go into induced ventricular fibrillation and a resistant canine will not. While there are many different cardiac diagnostic tools to obtain data to analyze the heart, few exist to diagnose arrhythmias, which may lead to VF. Only one currently exists to predict VF.

1.3 Cardiac testing tools

Currently there are many testing methods used to collect cardiac data. To name a few, methods used to collect data are stress testing, radioactive tracer tests, and echocardiography. From these data, tools can determine HRV, an index of cardiac vagal tone, coronary blood flow, and ischemia. While these symptoms may interrelate providing valuable data regarding the heart arrhythmias and the possibility of VF, they cannot measure changes in ventricular repolarization that have been linked to an increased risk of VF. The only test that does measure VF directly is the electrophysiological test.

To analyze the heart response to factors leading to VF, the current diagnostic tools can determine HRV, an index of cardiac vagal tone, but cannot measure changes in ventricular repolarization that have been linked to an increased risk for VF. Additionally, the current methods for measuring the risk of VF are not always reliable, are not without risk, and neglect important components of EKG that are explored in this thesis.

Current methods for collecting cardiac data during situations that can be pre-cursers to cardiac trauma are the stress test, echocardiography, radioactive tracers, and electrophysiological testing. The electrophysiology test is an invasive test that stimulates the heart by electrical impulses. The invasive surgery is simply the insertion of a catheter into the groin. The catheter is threaded to the heart, where it evaluates the hearts conductive properties. The catheter also delivers electrical impulses to the heart causing increased heart rate and minor arrhythmias. The test is designed to see how the heart responds to rhythm disturbance, a.k.a arrhythmias. The test is dangerous for two reasons. Firstly, any invasive procedure comes with risk, but an invasive procedure that forces arrhythmias to the heart is even riskier. The test is also expensive because of the personnel, equipment, and time needed during the procedure.

The radioactive tracer test details blood flow to the sections of the heart providing valuable information about infarct tissue, but does not provide information regarding the electrical impulses and action potentials that lead to VF. The tracer characterizes ischemia, which affects the electrical system in the heart, but does not directly assess arrhythmias. In addition to not classifying arrhythmias, the use of radioactive isotopes may have adverse health effects too.

The echocardiography test is essentially an ultrasound. This test provides information regarding the mechanical function of the heart and coronary blood flow. It is not used to diagnosis arrhythmia risk, but a well established association with left ventricular function and mortality has been tied to these test results. Patients with low

ejection fractions and stroke volume can be warned of these potential issues by the non-invasive procedure. Additionally, the diagnosis based on an echocardiography is dependent upon the physicians experience, knowledge, and keen eye of all which have a wide statistical variation.

The stress test, while being a good test for determining HRV and vagal tone, do not provide detailed information in assessing arrhythmia risk. The data collected from the stress test is used to test underlying coronary blood flow problems. Problems that may lead to ischemia in turn affecting the electrical system in the heart. Even though the test may provide some information, it has high false negatives, especially when diagnosing females.

1.4 Problem statement

Knowing ischemia induces higher heart rate and the potential for interrupting the electrochemical gradient needed to maintain the refractory period of the heart, ischemia produces prime conditions for VF. Knowing the T wave of an EKG measures the repolarization characteristic of the ventricle, searching for abnormalities and T wave variations between resistant canines and susceptible canines may provide detail information allowing for the pre-diagnoses of arrhythmias that lead to VF. Additionally, knowing the current T wave analysis methods, ones only focusing on the peaks of each wave, neglects important information and has not proved accurate or viable in analyzing

the risk of VF, it seems best to combine the concepts ischemia and full T wave analysis to determine a subjects risk to VF.

To combine the two concepts, analyzing the T wave of an EKG signal during four stages, control, occlusion, reactive hyperemia (recovery), and fully recovered can allow for the analysis between susceptible canines and resistant canines to determine if there are any variations useful in predicting the canine susceptibility or resistance to VF. It is hypothesized while the heart is experiencing ischemia, demonstrated in the occlusion stage, that variations will become more apparent between susceptible and resistant canines. This is what is to be determined this thesis.

1.5 Organization

In the following chapter, we discuss the methodologies used during the data collection procedures. The experiments used to analyze the data collected will be outlined too.

Chapter III presents the results of the experiments by searching for statistical significance in the experimentally processed data.

Finally, this thesis concludes with a summary and implications of the results, Chapter IV. Also, suggestions for possible research in the area are made.

CHAPTER II

METHODOLOGY

This section explores the methods used to collect data for analysis, the isolation of the necessary data, and the experiments used to analyze the isolated data.

2.1 Animal preparation and protocol for separation of groups

In order to perform a spectral T wave analysis as specified by the problem statement in Chapter I, an accurate set of data must be collected. The data are critically dependent upon the model used. The model must mimic, as closely as possible, the underlying pathological conditions associated with a high risk of sudden death. Studies indicate that among the most important factors associated with a high risk of sudden death, previous myocardial ischemic injury and infracted myocardium tissue are most important. The data pools analyzed are composed of canines prepared by surgery to mimic these ischemic pathological conditions.

To prepare the canine's pathological conditions, open-heart surgery is performed. During surgery, preventing blood flow to the anterior portion of the heart induces an anterior myocardial infarction. An occluder/flow monitoring device, here after referred to as the occluder, is then placed around the left circumflex artery. This occluder is later used manually to induce acute ischemia and monitor coronary blood flow. Inducing ischemia coupled with the previous infarct tissue generates the necessary components to cause VF.

Monitoring the blood flow through the artery is performed by a pulsed Doppler coronary flow transducer. The Doppler technique sends an ultrasonic pulse into the blood stream that is reflected off the red blood cells. The signal is shifted proportional to the blood flow velocity (i.e., the Doppler shift). EKG leads are also placed on the heart. Two sets of electrodes are sutured to the left and right ventricle to record the canine's EKG during testing.

Once the canines are fully recovered from surgery and trained to run on a motorized treadmill, EKG and CBF data are collected under two types of cardiac stress: rest and exercise. During rest EKG data and CBF data was taken for a total of 10 minutes. The first 3 minutes were to get a control EKG sample. The next 2 minutes where taken while the circumflex artery was occluded by the occluder and ischemia was induced. The next 5 minutes was post occlusion and can be broken down into the recovery stage and the recovered stage. The recovery stage immediately follows the occlusion release and the recovered stage is about 2 minutes after the occlusion release.

VF susceptibility vs. resistance for each canine is then determined by performing an exercise plus ischemia test. Animals will run 15-18 minutes with work load increasing every 3 min until a heart rate of 210 beasts/min is achieved. During the last minute of exercise the left circumflex occluder is inflated, the treadmill is stopped and the occlusion maintained for an additional minute. The total occlusion length is 2 minutes: 1 minute during exercise and 1 minute post exercise. This allows for the differentiation of arrhythmias induced during exercise, post exercise, and post occlusion release. The

occlusion is immediately released in those animals that exhibit ventricular tachyarrhythmia that most frequently becomes ventricular flutter that rapidly deteriorates into VF determining a canine's susceptibility to VF.

The recording of the coronary blood flow (CBF) is an important step. While data are being collected the occluder is inflated to induce ischemia. Ischemia, as mentioned before, is important because it creates an environment primed for VF by effecting HRV and electrochemical gradients. Not only does it increase heart rate but it also blocks blood flow to the myocardium. If the myocardium does not receive blood then the channels controlling the electrochemical gradient are unable to function therefore preventing proper action potentials leading to ventricular tachycardia and/or VF. For these reasons the EKG signal and the CBF through the left circumflex is monitored during 4 stages at rest and exercise: Control, Occlusion, Recovery, and Recovered. These fours sections will be explained in more detail later. For this thesis, the EKG signals and CBF signals of resistant and susceptible canines were examined from the 10 minute rest period.

2.2 T wave isolation

In order to perform a spectral T wave analysis as specified by the problem statement in Chapter I, EKG and CBF data are collected from 14 pre-identified susceptible or resistant canines. (susceptible, n=8; resistant, n=6). Each subject's EKG was then subdivided into four epochs: the control epoch, occlusion epoch, recovery epoch, and the recovered epoch.

The control epoch of the data is used as a control signal for each canine subject. This is the 3 minute rest period before occlusion. The occlusion section of the CBF represents ischemia, one epoch of interest. During the occlusion section, the occluder is manually inflated to block blood flow through the circumflex coronary artery causing an ischemic response in the heart. The recovery section occurs when the occluder is released allowing blood to flow back to the heart. Finally, the recovered section represents the CBF stabilizing and providing a steady flow of blood.

2.2.1 Epoch definition from data

In order to properly analyze the T wave of the EKG during each epoch condition the corresponding epoch section of data must be accurately located. Locating these points was done by analyzing the CBF. The control epoch and the recovered epoch were easily isolated since they are the first 60000 and last 60000 data points of the EKG and the CBF data. To locate the start and stop point of the occlusion, windowing threshold detection was used. As shown in Figure 4, the starting position of the occlusion was found when the maximum value of the data points in the windowed data was less than 0.5V and the stop point was when the maximum value in the windowed data was greater than 0.5V. The value 0.5V empirically determined and varied subject to subject. The window size was determined as the maximum distance between two consecutive QRS signals in the EKG data, and the threshold of 0.5volts was chosen by visually inspecting the CBF to see what values corresponded to a drop in blood flow. Threshold varied from animal to animal. Some CBF data showed the occlusion section rose instead of dropped.

This was simply caused by reversing the connecting wires causing the CBF output signal to be inverted.

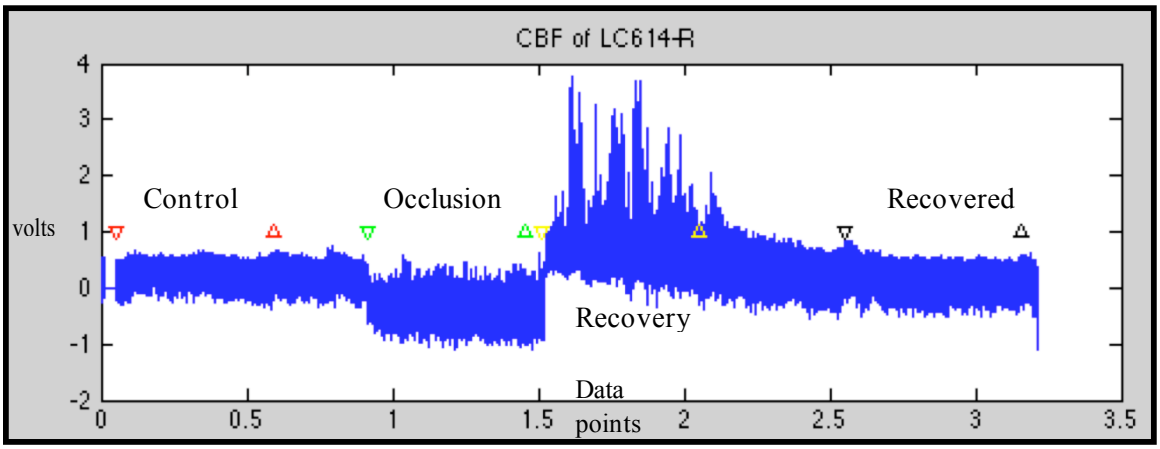

Figure 4: Coronary Blood Flow (CBF) sectioned into epoch zones

Once the occlusion start location was determined, data were taken for 60000 points (2 minutes). This 2 minute section captured the entire occlusion section, because a standard protocol was used when capturing data - 3 minute control, 2 minute occlusion, 5 minutes post occlusion. Finally, since the end occlusion data point was previously found, the recovery section was easy to isolate too. The final stage was to transfer the timing of epochs found in the CBF to the EKG signals. Since the sampling rate for the CBF was the same as the EKG's, locating the epochs on the EKG was a simple transferred from the CBF to the EKG as shown in Figure 5.

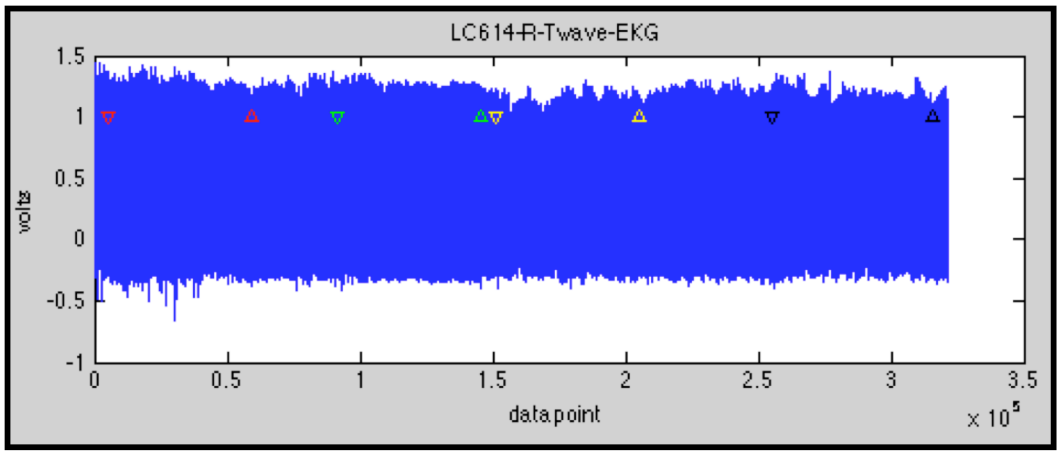

Figure 5: EKG sectioned into epoch zones

2.2.2 Selection of T wave windowing

The next step in the data collection procedure is to locate the T wave. Locating the T wave can be difficult, but finding the QRS complex is simple. Since the QRS is a dominant component of the EKG and has a much larger peak than other values it can be found. A match filter technique using an ideal QRS signal and convolving it with each EKG section was used to determine the location of each QRS segment. Then using threshold detection, on the filtered EKG, the index locations of the QRS were found. A four-part picture showing the expanded sections of the EKG to be analyzed, the ideal-QRS, the convolution, and the QRS detection is shown in Figure 6.

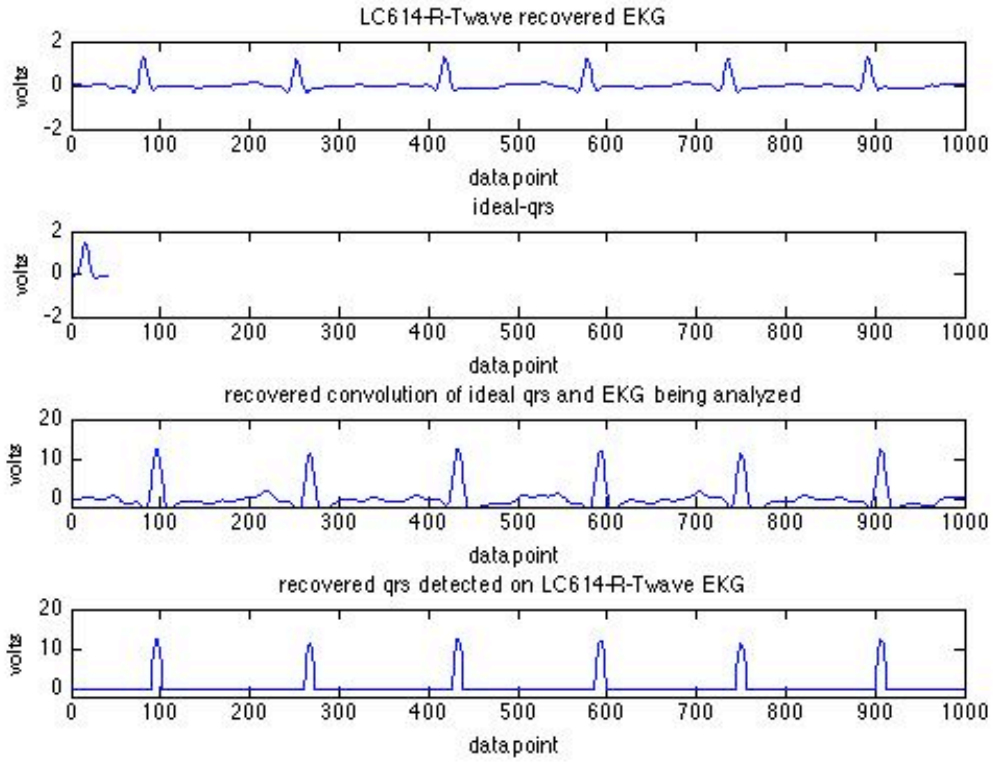

Figure 6: QRS detection. Zoomed in portion of EKG to be analyzed (top), ideal-QRS (middle top), zoomed in portion of convolution of ideal-QRS and EKG (middle bottom), QRS detected (bottom)

Once the QRS location is detected the T wave windows can be determined. T waves at times are very difficult to detect not to mention the T wave variations being explored by this thesis. To capture the T wave, a delay is implemented after the QRS complex. The delay, typically 15 data point or 30 ms, was chosen empirically for each subject, so that a T wave isolation window did not include any part of the QRS complex.

The T wave isolation window is a 64 data point (128ms) wide window. The window size was chosen for two reasons. Firstly it is large enough to include the entire T wave while ignoring unwanted parts of the EKG like the P wave. Secondly the window size was chosen to be a power of 2 ($2^p = 64$, p=6) because the properties of Fourier transforms

calculate quicker for powers of 2. Another beautiful aspect of the FFT properties is that even though the T wave window consisted of 64 data points, only 32 need to be analyzed and viewed because the FFT is symmetric. Thus if data point 64 is the same as data point 1, there is no need to look at them both since one will tell use the same information as both.

The use of the FFT is to convert the time domain T wave data into the frequency domain allowing for spectral analysis. Data in the frequency domain allows for an easier analysis of smooth and abrupt changes seen in the time domain. Converting to the frequency domain takes gradual slope changes in a T wave and characterizes them to low frequencies and sharp slope changes are characterized to high frequencies. This allows for numerical interpretation of T wave changes. These frequencies can then be analyzed in terms of magnitude or phase.

The phase portion of the spectral T wave data is of no interest in this study. The phase is ignored because, as said before, all canines have a different pause needed to isolate the T wave. Additionally, to assure the data being analyzed were not corrupted by baseline (DC) drift, 60 Hz signal from the wall outlets or any of 60Hz harmonics, these values were removed from the T wave spectral data. In order to analyze the magnitude portion of the T wave, the absolute value (ABS) of the T wave frequency spectrum is taken.

2.2.3 Noise reduction in epoch spectra

The last step in preparing the spectral T wave data is to remove excess noise in the signal. Each epoch contains approximately 400 T waves. Removing excess noise is accomplished by averaging each frequency of the spectrum over all cardiac cycles in the epoch. Figure 7 shows the T wave spectral data before and after averaging across cardiac cycles.

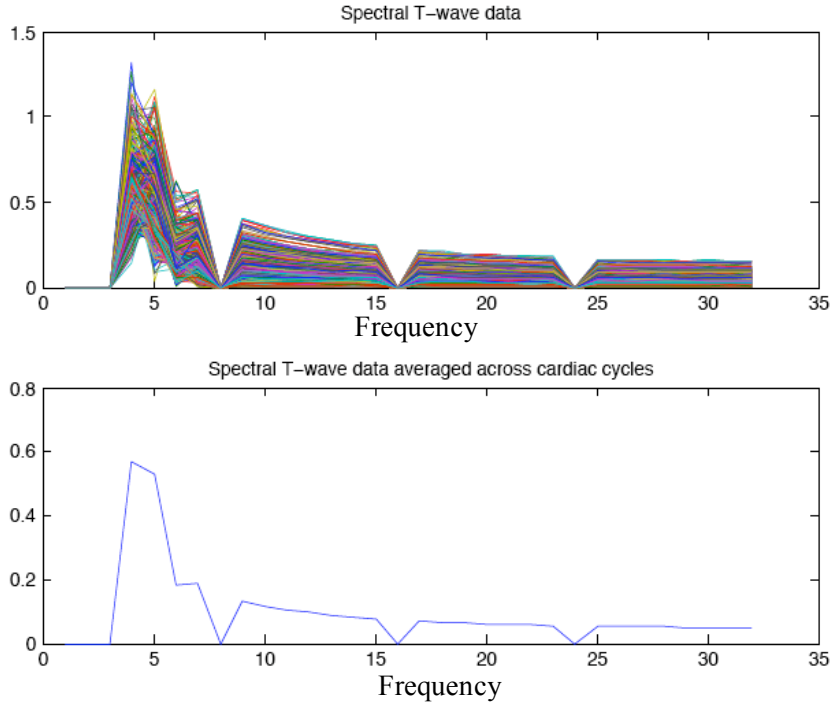

Figure (7): Spectral T wave data (top); Spectral T wave data averaged across cardiac cycles (bottom)1

2.3 Experiments

The concepts of both section 2.1 and section 2.2 were applied to a set of 14 canine EKG traces. 6 of the 14 traces were collected from VF resistant canines and the other 8 were collected from VF susceptible canines. The following experiments were applied to the data.

2.3.1 Experiment 1

Keeping in mind that t wave spectral differences between resistant and susceptible canines are hypothesized as more apparent during ischemia, the root mean square (RMS) difference between full spectrum of occlusion, recovery, and recovered epochs vs. the control epoch, were studied. The data manipulation to perform this RMS comparisons were as follows: To normalize the data, the averaged full spectrum T wave in each of the occlusion recovery, and recovered epochs were subtracted from the averaged control spectral epoch. This subtraction allowed each animal to have its own control T wave spectrum. After subtraction, the frequency samples for each animal where squared, averaged and then the square root taken for each epoch. The RMS was computed for the variations of each epoch comparison for resistant and susceptible canines, and finally a 2 sample ttest with different variances was performed comparing resistant and susceptible canines using Matlab.

The ttest2 function on Matlab assesses whether means of two groups are statistically different from each other. The null hypothesis is that the means are the same. In context to the EKG data being analyzed, the objective is to reject the null hypothesis indicating that there is a difference between the susceptible and resistant T wave RMS spectral means. The ttest2 function returns the values H, P, and Ci. H is the acceptance or rejection of the null hypothesis of the probability of observing a value with the same mean (H = 0 acceptance the null, H=1 rejects the null hypothesis). P is the significance of data, the probability, under the null hypothesis, of observing more extreme test statistics.

A P value less than 0.05 or 5% will reject the null hypothesis and is what this thesis seeks. Ci is the confidence level.

To analyze the RMS data of the full spectral T wave data, it is broken down into two tables. Table 1 shows the data for the spectral T waved data for resistant canines and Table 2 shows the spectral T wave data for susceptible canines.

RMS Resistant T-wave Spectral analysis

Canine ID	Occlusion epoch	Recovery epoch	Recovered epoch
614	0.1444	0.0406	0.0338
697	0.2148	0.0671	0.0277
738	0.08	0.0242	0.0223
761	0.0203	0.0063	0.0088
674	0.2573	0.0622	0.0456
725	0.043	0.0384	0.0276

Table 1: RMS of spectral T wave epoch comparison data for resistant canines

RMS Susceptable T-wave Spectral analysis

Canine ID	Occlusion epoch	Recovery epoch	Recovered epoch
617	0.0357	0.0467	0.015
699	0.1777	0.0969	0.0516
722	0.0281	0.0499	0.0216
734	0.0315	0.0091	0.0085
764	0.2771	0.1737	0.0792
691	0.673	0.6814	0.4525
778	0.1198	0.0584	0.0837
784	0.1059	0.0669	0.0354

Table 2: RMS of spectral T wave epoch comparison data for susceptible canines

These comparisons were visually analyzed for trends and variations. Figure 8, below, shows the averaged spectral T wave epoch for each resistant and susceptible canine.

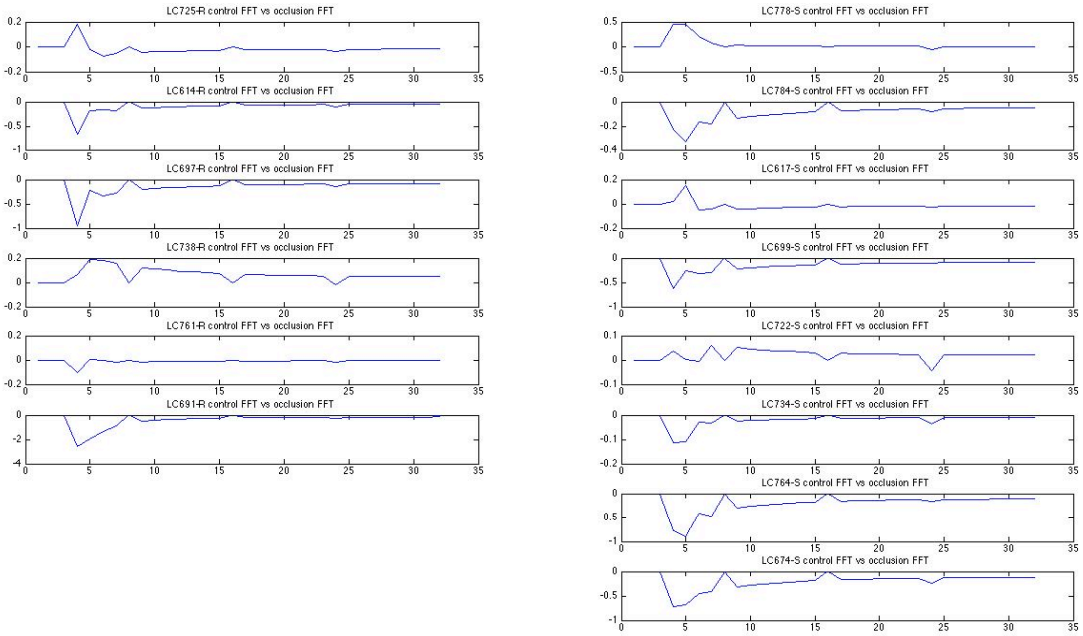

Figure 8: Averaged Spectral T-wave control epoch vs. occlusion epoch for tested subjects

2.3.2 Experiment 2

Experiment 2, is almost identical to Experiment 1. There is, however, an extra step. Before taking the RMS of the full spectral data, the T wave spectral data is segmented into four sections: High, Mid-High, Mid-Low, and Low frequencies with each section being composed of about 70Hz. Figure 9, shows the segmented averaged spectral T wave control epoch vs. the averaged spectral T wave occlusion epoch for each resistant and susceptible canine.

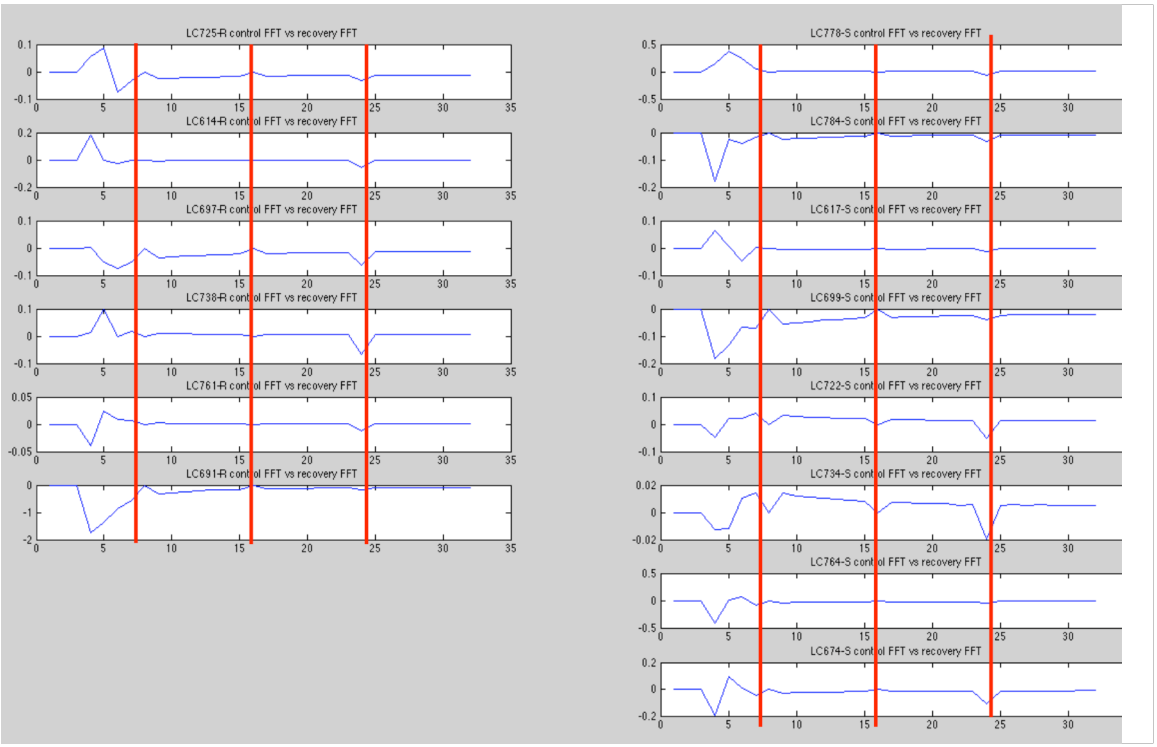

Figure 9. Segmentation of T wave spectrum into 4 sections for concentrated analysis.

The RMS of each section for each epoch comparison was computed for both resistant and susceptible canines, to determine variations for each epoch comparison, as shown in Tables 3, below.

RMS of sectioned Resistant T-wave Spectral analysis

Canine ID	Segment 1 occlusion epoch	Segment 2 occlusion epoch	Segment 3 occlusion epoch	Segment 4 occlusion epoch	Segment 1 recovery epoch	Segment 2 recovery epoch	Segment 3 recovery epoch	Segment 4 recovery epoch	Segment 1 recovered epoch	Segment 2recovered epoch	Segment 3 recovered epoch	Segment 4 recovered epoch
614	1.7814	0.6694	0.4964	0.3633	1.7774	0.5345	0.6842	0.2951	1.8876	0.1803	0.6276	0.1021
697	1.7596	0.7138	0.4948	0.3867	1.8155	0.5819	0.5144	0.3177	1.3268	0.9795	1.0001	0.5292
738	1.3801	1.1106	0.7016	0.6079	1.4842	0.7566	1.0296	0.4054	1.6167	0.3322	1.1148	0.1814
761	1.8149	0.6001	0.4963	0.3157	1.8612	0.0394	0.7304	0.0317	1.9323	0.1124	0.5018	0.0427
674	1.6044	0.8889	0.6356	0.4815	1.3891	0.9418	0.9581	0.5152	1.6969	0.464	0.9146	0.2622
725	1.6978	0.7651	0.5865	0.4336	1.9783	0.1359	0.2407	0.0994	1.6904	0.7391	0.6378	0.4353

RMS of sectioned Susceptable T-wave Spectral

Canine ID	Segment 1 occlusion epoch	Segment 2 occlusion epoch	Segment 3 occlusion epoch	Segment 4 occlusion epoch	Segment 1 recovery epoch	Segment 2 recovery epoch	Segment 3 recovery epoch	Segment 4 recovery epoch	Segment 1 recovered epoch	Segment 2recovered epoch	Segment 3 recovered epoch	Segment 4 recovered epoch
617	1.6184	0.8858	0.6088	0.4748	1.9946	0.0894	0.1049	0.049	1.9186	0.3669	0.3782	0.203
699	1.5911	0.919	0.612	0.4993	1.7094	0.7882	0.5296	0.4196	1.6845	0.8037	0.5733	0.4336
722	0.888	1.3086	0.9963	0.7115	1.3444	1.1275	0.7389	0.6126	1.1541	1.0536	1.1019	0.5864
734	1.8174	0.5712	0.5271	0.305	1.7266	0.0774	1.0053	0.0464	1.0245	1.1832	1.0797	0.6201
764	1.7169	0.7798	0.5199	0.4169	1.9781	0.2099	0.1747	0.1117	1.9144	0.3958	0.3576	0.2248
691	1.8938	0.5143	0.3055	0.2364	1.9046	0.4879	0.29	0.224	1.911	0.47	0.2859	0.2132
778	1.9846	0.1616	0.1732	0.0725	1.8989	0.4025	0.4589	0.1463	1.9838	0.0149	0.2537	0.0028
784	1.5676	0.9429	0.6335	0.5024	1.9544	0.3035	0.2529	0.1561	1.8694	0.5024	0.4339	0.2539

Table 3: RMS Spectral T wave data of segmented data

Finally a 2 sample ttest with different variances was performed comparing resistant and susceptible canines. The results are discussed in Chapter 3.

2.4 FAILED EXPERIMENTS

One other, unsuccessful experiment performed on the data comparing the T wave spectral alternans. Traditional alternan analysis compares the T wave peaks (max value) of consecutive T waves and analyzes for variations like sinusoidal rhythms. It is hypothesized if such variations exist then a canine will be susceptible to VF.

T wave alternans as pertaining to this thesis is comparison of even and odd T waves. To separate and compare the alternans T wave, data were separated into even and odd groups. The odd group was composed of the 1^{st}, 3^{rd}, 5^{th}….T wave found in the EKG data, while the even group was composed of the 2^{nd}, 4^{th}, 6^{th}….T wave found in the EKG data. These two vectors were subtracted yielding alternan spectral T wave data in addition to the spectral T wave data. The advantage to analyzing T wave alternans, as defined, is the removal of baseline drift from the spectrum signals. The results of this experiment were not statistically significant because no patterns were observed.

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CHAPTER III

RESULTS ANALYSIS

This chapter reports the results of experiment 1 and experiment 2, previously discussed in Chapter 2.

3.1 ttest2 statistical analysis

The subject pool for this thesis originated with 5 canines susceptible to VF canines and 4 canines resistant to VF. After processing the RMS of the spectral T wave epoch comparison data using the ttest2 function, described in section 2.3, the null hypothesis was accepted. The results of the ttest2 operation can be seen in Table 4.

ttest2 results of 6 RMS Resistant canines compared to 8 RMS susceptible canine T-wave Spectral data

	Occlusion Epoch	Recovery Epoch	Recovered Epoch
H:	0	0	0
P:	0.4951	0.5612	0.7353
C:	-0.0021	-0.0038	-0.0033
	0.004	0.0023	0.0025

Table 4: ttest2 results of RMS resistant compared to RMS susceptible T wave Spectral data

The null hypothesis was not rejected because of the high p-values returned by the ttest2 function. To reject the null hypothesis, determining variations between resistant and susceptible T wave spectral epochs, the p-values of each epoch section need to be less then 0.05 or 5%.

Since analyzing 3 susceptible and 2 resistant canines failed to reject the null hypothesis and returned high p-values, the subject pool was increased to 8 susceptible and 6 resistant canines with hopes of lowering the p-value and in turn rejecting the null hypothesis. The RMS for the T wave epoch comparison data, shown in Tables 1 and 2, was processed with the ttest2 function with Table 5 showing the results.

ttest2 results of 6 RMS Resistant canines compared to 8 RMS susceptible canine T-wave Spectral data

	Occlusion Epoch	Recovery Epoch	Recovered Epoch
H:	0	0	0
P:	0.5401	0.2108	0.2494
C:	-0.1365	-0.0769	-0.0578
	0.2453	0.293	0.1895

Table 5: ttest2 results of 6 RMS resistant canines compared to 8 RMS susceptible canines T wave Spectral data

The new ttest2 results of the larger sample data yielded a promising outcome. Even thought the null hypothesis still fails to be rejected, the p-values with larger groups, has dropped greatly. The occlusion epoch p-value differed from 0.4951 to 0.5401, and the recovery epoch p-value dropped from 0.5612 to 0.2108, and the recovered epoch p-value dropped from 0.7353 to 0.2494. The recovery and recovered epoch data gave very promising results that with larger samples a rejection of the null hypothesis maybe possible and reinforces that looking at the recovered and recovery epochs may provide more valuable data than the ischemic section as originally thought. But the null hypothesis was still accepted.

Still needing to reject the null hypothesis, the steps outlined in Experiment 2 were explored. Knowing each T wave is composed of frequencies ranging high to low, it is hypothesized that sub-dividing these frequencies into groups and analyzing the ranges separately would locate a section in the T wave spectrum enabling the ttest2's function to reject the null hypothesis. The results of the ttest2 function are outlined in Table 6.

ttest 2 results of the segmented RMS valuse of the Resistant T waves compared to the segmented RMS values of the Susceptable T waves

	Segment 1 Occulsion Epoch	Segment 2 Occulsion Epoch	Segment 3 Occulsion Epoch	Segment 4 Occulsion Epoch	Segment 1 Recovery Epoch	Segment 2 Recovery Epoch	Segment 3 Recovery Epoch	Segment 4 Recovery Epoch	Segment 1 Recovered Epoch	Segment 2 Recovered Epoch	Segment 3 Recovered Epoch	Segment 4 Recovered Epoch
H:	0	0	0	0	0	0	0	0	0	0	0	0
P:	0.7837	0.8328	0.823	0.7258	0.4455	0.7509	0.1495	0.5901	0.955	0.5125	0.1497	0.5986
C:	-0.3394	-0.3455	-0.2319	-0.207	-0.1724	-0.4849	-0.601	-0.281	-0.3604	-0.2926	-0.5832	-0.1778
	0.2628	0.2837	0.1889	0.1488	0.365	0.3598	0.1041	0.1676	0.342	0.5544	0.1003	0.2946

Table 6: ttest results of segmented T wave spectrum.

Again, as before, the null option has not been rejected and the variations of the susceptible and resistant spectral T wave RMS data overlaps too much to significantly see variations between resistant canine and susceptible canine groups. However, just as before, noticing the probabilities listed in Table 6, the p-values correlating to segment 3 of both the recovery epoch and the recovered epoch have dropped closer to the desired value of 0.05. This drop even closer to 0.05, shows promise that a continued segmentation of the averaged T wave spectrum may yield results to reject the null hypothesis of the ttest2 function.

3.2 Number of subjects needed to reject null hypothesis

With the ttest2 showing a very strong trend toward a 5% probability, this leads to the question: How many resistant canines and susceptible canines are needed to reject the null hypothesis. Looking at the concept of Experiment 1, increasing the subject data pool, and

using the equation below to determine the sample size needed to reject the null hypothesis of two normally distribute samples of equal size with different variance. [5]

$$\text{sample size} = n = \frac{(\sigma_1^2 + \sigma_2^2)*(Z_o + Z_1)^2}{\Delta^2} \quad \text{Equation 1}$$

n = the appropriate sample size in each group to have a probability of 1-β (β=5%) of finding a significant difference in the true differences in means between groups.

Δ = Difference between the mean of the resistant canine RMS values and the susceptible canine RMS values.

σ = The standard deviations of two respective samples, in this case we estimate the standard deviations with the variances of the resistant canine RMS values and the susceptible canine RMS values.

Z_0 = 1.645 and Z_1 =1.96. These values are given by the standard Gaussian distributions and are obtained by simply looking the values up. [5]

Plugging in the appropriate values for the recovery epoch section of analysis, seen below, yields a value of n=60.3.

$$n = \frac{((0.0246)^2 + (0.221)^2)*(1.96 + 1.645)^2}{(0.148-0.0448)^2} \quad \text{Equation 2}$$

Performing the same operation with the recovered epoch analysis yields a value of n = 74.8. This equation shows that to reject the null hypothesis of the ttest2 function, approximately 120 total subjects, 60 animals in each group, will be needed for the

recovery epoch analysis and, 150 total subjects, 75 animals in each group, will be needed for the recovered epoch analysis.

Another important equation that can be used to determine the number of animals needed to reject the null hypothesis, factors in the standard deviations of the data being analyzed. As seen in equation 3, the standard deviations for the susceptible case - 0.221 - is about 10 times larger than the standard deviation for the resistant case – 0.0246. Using another equation that determines the sample size needed for comparing the means of two normally distributed samples of unequal size, shown below, the number of EKG traces can be reduced. [5]

$$\text{Sample size} = n_1 = \frac{(\sigma_1^2 + \sigma_2^2/K)*(Z_o + Z_1)^2}{\Delta^2} \qquad \text{Equation 3}$$

And

$$\text{Sample size} = n_2 = \frac{(K*\sigma_1^2 + \sigma_2^2)*(Z_o + Z_1)^2}{\Delta^2} \qquad \text{Equation 4}$$

All variables are the same as before except for k. K is the ratio of standard deviations. In this case K = 10. Applying values to the equation for the recovery epoch analysis shows that 8 resistant EKG traces and 35 susceptible EKG traces are needed to reject the null hypothesis. For the recovered epoch analysis 9 resistant EKG traces and 82 susceptible EKG traces are needed to reject the null hypothesis.

Equation 3 and 4 show that factoring the standard deviations ratio into account, when determining the number of animals needed to reject the null hypothesis, is beneficial

when analyzing the recovery epoch since 8 + 35 = 43 which is less than using the equation that assumes equal data sets - equation 2. Regarding the recovered epoch analysis, using an equal sample size would be less beneficial because it would require 150 animals compared the 82 + 9 = 91 traces calculated using equation 3 and 4.

Applying the same equations and concepts mentioned before to Experiment 2,it would take a total of 37.5 animals, 19 resistant and 19 susceptible, to reject the 2 variable ttest null hypothesis of the recovery epoch in segmented zone 3. To reject the null hypothesis of the recovered epoch in segmented zone 3, it would take a total of 39.4 animals, 20 resistant and 20 susceptible. The benefits of segmenting the spectral epochs, in addition to lower the p-values, is that it allows for a smaller subject pool required to reject the null hypothesis, thus showing statistical significance when finding variations between T wave spectrums of susceptible and resistant canine groups.

3.3 Difficulties

Some difficulties encountered with obtaining the number of traces needed to reject the null hypothesis, is that the data being analyzed, both CBF and the EKG, need to be in good condition. During the data acquisition stages, some EKG where filled with too much noise and the CBF did not always mark an accurate occlusion section. The EKG noise made it difficult to locate the T wave, while the inaccurate CBF occlusion section made it impossible to locate the epochs to be analyzed. For this reason it was difficult to locate data.

CHAPTER IV

CONCLUSION

In this chapter we discuss the conclusions that can be drawn from the data presented in the previous chapter. In addition, some discussion about possible extension of this study is included.

4.1 Conclusion

Based on data presented and arguments made in the last chapter, that this method of analysis shows promise in distinguishing VF resistant vs. susceptible animals. Provided the p-value trends continue as segmentation of the averaged T wave spectra RMS, the null hypothesis of the ttest2 should reject if enough animals are used n the study. This rejection would verify variations between the T wave spectrum of canines resistant to ventricular fibrillation and those susceptible to ventricular fibrillation.

At the thesis inception, the method was to analyze the T wave spectrum of pre-identified canines during the ischemia. This ischemia was represented by the occlusion of the left circumflex coronary artery. After statistically analyzing the averaged T wave spectrum root mean square, it was determined that analyzing the epochs of a recovery section after an occlusion to the left circumflex artery, and the recovered epoch after the occlusion would prove more statistically valuable.

Additionally, segmenting the T wave spectrum into high, mid-high, mid-low, and low sections, showed promise toward statistical significance. Segmenting the T wave allows for a more accurate comparison of T wave variations between canine susceptible and resistant to ventricular fibrillation. These comparisons of T wave spectral variations show that by analyzing the T wave in the frequency domain, it can be shown a canine is susceptible or resistant to ventricular fibrillation

4.2 Summary and extensions

In this thesis, we have presented a method for determining the susceptibility or resistivity of a canine to ventricular fibrillation by analyzing the T wave spectrum of an EKG. While the process was not yet proven to have statistical significance, the theory is encouraging.

Further work in this area might implement further spectral segmentation of the T wave. This would allow for a finer analysis of the T wave and the ability to more accurately isolate the T wave spectral range responsible for defining the spectral T wave variation indicative of canines resistive or susceptive to ventricular fibrillation.

While attempting the general solution, mentioned above, several details were set in order to complete the project in a timely fashion. However, if these can be generalized as well, the routine may have even broader applications. One example would be to analyze the P-wave spectrally to determine if atria arrhythmias can be pre-diagnosed. Another example would not ignore the phase shift of the t-wave with the assumption that data may be hidden in this spectrum too. However, it is clear that these are still drawbacks,

inefficiencies, and isolation shortcomings. Only future work can build upon this process in an attempt to more accurately determine ventricular susceptibility or resistivity to ventricular fibrillation in an in vivo canine model.

4.3 Training Principles

This thesis acted as an excellent educational tool. Not only did it develop Matlab coding skills, but it also demonstrated a practical application to the theories taught through out my engineering career. This thesis allowed for a project that was a personal education tool, bridging engineering and medicine; two areas of extreme personal interest of engineering and medicine. The opportunities to observe and assist in open heart surgery provided first hand exposure to the field of medicine, while the development and analysis of the collected data provided and engineering aspect. Additionally, practical statistics were utilized allowing me to see the significance of my data, oppose to simply trusting theory and a professors word that the math behind the theory works. Most importantly, the thesis allowed for an interdisciplinary project that not only involved knowledge of multiple fields, but required the development of communications skills. Learning how to talk amongst engineers and then translate information into a physician language proved to be and will continue to be a valuable asset.

BIBLIOGRAPHY

[1] Billman, G.E., C. (2006) A comprehensive review and analysis of 25 years of data from an in vivo canine model of sudden cardiac death: implications for future anti-arrhythmic drug development. *Pharmacology & Therapeutics* 111 (2006) 808-835.

[2] E. Widmaier, R. Hershel, K. Strang, *Human Physiology, tenth Ed.* McGraw-Hill, 2006.

[3] Polar RS800 – Polar RS800SD heart rate monitor[internet]. BHIP lrs, 2006 [Cited 2008 May 17]; Available from: http://www.heartratemonitor.co.uk/Manuals/RS800/ch11.html

[4] Hohnloser S. M.D., Kirk M. M.D., et. al. Non-invasive test to predict sudden cardiac death proven effective. American Heart Association [internet]. 2006 [Cited 2008 May 17]; Available from: http://www.americanheart.org/presenter.jhtml?identifier=3043452

[5] Rosner, Bernard. *Fundamentals of Biostatistics.* PWS Publisher, 1986.